**2006 ERSD Annual Report**

*DOE-BER Environmental Remediation Sciences Project # 1003686*

# Subsurface Biogeochemical Heterogeneity (Field-scale removal of U(VI) from groundwater in an alluvial aquifer by electron donor amendment)

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**Research Objective**: Determine if biostimulation of alluvial aquifers by electron donor amendment can effectively remove U(VI) from groundwater at the field scale. Uranium contamination in groundwater is a significant problem at several DOE sites. In this project, the possibility of accelerating bioreduction of U(VI) to U(IV) as a means of decreasing U(VI) concentrations in groundwater is directly addressed by conducting a series of field-scale experiments. Scientific goals include demonstrating the quantitative linkage between microbial activity and U loss from groundwater and relating the dominant terminal electron accepting processes to the rate of U loss. The project is currently focused on understanding the mechanisms for unexpected long-term (~2 years) removal of U after stopping electron donor amendment. Results obtained in the project successfully position DOE and others to apply biostimulation broadly to U contamination in alluvial aquifers.

**Research Progress and Implications:** This report summarizes results of four prior field experiments (2002 to 2005) and one field experiment in progress (2006 extending into 2007). The Rifle, Colorado uranium mill tailings site has a shallow alluvial aquifer with a well-defined porous medium flow system that is ideal for field-scale electron donor amendment for uranium bioreduction. Key results from these experiments are summarized as follows:

- Biostimulation removes U(VI) from groundwater under field conditions in a permeable alluvial aquifer.
- Direct microbial reduction by Geobacter *sp.* is largely responsible for U(VI) loss during the Fe-reduction phase of field experiments.
- Maintenance of Fe-reducing conditions is critical to ongoing removal of U(VI) during biostimulation.
- However, long-term (~2 years) post-amendment removal of U(VI) occurs when significant sulfate reduction was achieved during amendment.

- Post-amendment removal of U(VI) may be microbially mediated, an hypothesis that is currently being tested in the 2006 field experiment.
- Geophysical techniques, especially complex resistivity and self potential measurements provide effective techniques for minimally invasive monitoring of biostimulation and post-biostimulation *in situ* processes (see project lead by Susan Hubbard, LBNL).

These results suggest that bioremediation of U(VI) is possible even in high-permeability alluvial aquifers. Sulfate reduction may not contribute to direct U(VI) bioreduction, but may be used to advantage to sustain biosorptive U(VI) removal after ending electron donor amendment. Significant opportunity exists to tailor in situ processes to achieve U(VI) removal and sequestration. For example, it should be possible to add sulfate to low sulfate systems if precipitation of sulfide proves to be a critical factor in long-term control of U(VI) concentration in groundwater.

The experimental approach used at the Rifle site is to introduce the electron donor at low concentrations under natural gradient conditions. Experiments conducted since 2002 have consistently replicated loss of U(VI) from groundwater synchronous with growth of Geobacter *sp.* after amendment of the subsurface with acetate targeted at *in situ* concentrations of either ~3 mM or ~10 mM. Electron donor amendment and associated Geobacter growth dominates the system until Fe reduction consumes bio-available Fe(III) solids, eventually limiting Geobacter growth. Because there is ~ 6 to 10mM sulfate in the groundwater, sulfate reduction dominates the system after bio-available oxidized Fe is consumed, resulting in extensive precipitation of amorphous FeS and calcite, particularly near the point of injection and in the deeper parts of the aquifer. Typically, U(VI) concentrations rebound with the onset of extensive sulfate reduction, but, remarkably, after acetate amendment is stopped, U(VI) concentrations fall again and can remain at ~20% of background levels for >1 year. Our current hypothesis is that the extent of post-amendment U(VI) removal is correlated with the amount of FeS precipitated. However, recent laboratory studies (N'Guessan et al. 2006.) indicate that $FeS_{0.9}$ cannot reduce U abiotically in these sediments suggesting that a microbial community different from either the Fe or sulfate reducers produces biopolymers that preferentially sorb U(VI) or that newly-formed Fe(III) oxides sorb U(VI). We are currently testing this hypothesis at the field scale by comparing side-by-side experimental plots, one in which sulfate reduction is allowed to continue for approximately two months and another in which sulfate reduction is limited to approximately three weeks (figure 1). Post-amendment comparison of U(VI) removal is expected to show that the experimental plot with little sulfate reduction will rebound to background U(VI) concentrations within a few weeks, whereas the plot with extensive sulfate reduction will show removal of U(VI) for at least several months after acetate amendment was stopped. Post-amendment groundwater and sediment sampling will enable us to assess both microbial community and mineralogy changes associated with ongoing U(VI) removal.

## Figure 1. Visual evidence of Fe(III) vs. sulfate reduction in cross-well mixing tubes.

***Fe reduction (pink color, absence of black ppt.)***

***Sulfate reduction (black is sulfide, likely FeS (+calcite)***

Geophysical measurements (complex resistivity, self potential, cross-well radar, and cross-well seismic) are providing minimally-invasive monitoring of both initial heterogeneity of redox status and microbially mediated changes in subsurface mineralogy and redox status. Initial complex resisitivity data from earlier experiments indicate that both changes in Fe-bearing mineral coatings on detrital grains and in sulfide mineral abundance can be tracked in 3-D, enhancing our ability to interpret groundwater and sediment geochemical changes and ultimately providing a means for real-time monitoring of uranium bioremediation.

1. **Planned Activities:** The current experiment will continue with post-amendment groundwater monitoring, sediment sampling, and geophysical monitoring through at least July of 2007. Based on results through that date and availability of funds, monitoring may continue into FY-2008. Significant additional experimental opportunities exist for the site and are the subject of a pending proposal and likely additional future proposals. Key areas for future field experiments include:

- Extension of Fe-reducing conditions.
- Impact of reducing conditions on U(VI) sorption.
- Post-biostimulation U(IV) stability and U(VI) removal.
- Rates of natural bioreduction.
- Predicted vs. observed response to electron donors other than acetate.
- Impacts of spatially and temporally variable site conditions on bioremediation efficacy.

2. **Information Access:** See http://www.pnl.gov/nabir-umtra/ for additional project information, especially http://www.pnl.gov/nabir-umtra/pubs.stm for published abstracts, book chapters, and theses in addition to the peer-reviewed journal articles listed below. An additional three journal articles are currently in review. Peer-reviewed journal articles below include those using site experimental data or materials and those based on research at UMTRA sites prior to the Rifle field experiments.

Chandler, D. P., A. E. Jarrell, E. R. Roden, J. Golova, B. Chernov, A. D. Peacock, and P. E. Long. 2006. Suspension array analysis of 16S rRNA from $Fe^{-2}$ and $SO_4^{-2}$ - reducing bacteria in uranium contaminated sediments undergoing bioremediation. Applied and Environmental Microbiology Vol. 72(7):4672-4687

Vrionis, H. A., R. T. Anderson, I. Ortiz-Bernad, K. R. O'Neill, C. T. Resch, A. D. Peacock, R. Dayvault, D. C. White, P. E. Long, and D. R. Lovley. 2005. Microbiological and geochemical heterogeneity in an in situ uranium bioremediation field site. Applied and Environmental Microbiology 71:6308-6318.

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Holmes, D. E., K. P. Nevin, R. A. O'Neil, J. E. Ward, L. A. Adams, T. L. Woodard, H. A. Vrionis, and D. R. Lovley. 2005. Potential for quantifying expression of the Geobacteraceae citrate synthase gene to assess the activity of Geobacteraceae in the subsurface and on current-harvesting electrodes. Applied and Environmental Microbiology 71:6870-6877.

Chang, Y. J., P. E. Long, R. Geyer, A. D. Peacock, C. T. Resch, K. Sublette, S. Pfiffner, A. Smithgall, R. T. Anderson, H. A. Vrionis, J. R. Stephen, R. Dayvault, I. Ortiz-Bernad, D. R. Lovley, and D. C. White. 2005. Microbial incorporation of C-13-labeled acetate at the field scale: Detection of microbes responsible for reduction of U(VI). Environmental Science & Technology 39:9039-9048.

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Nevin, K. P., K. T. Finneran, D. R. Lovley. 2003. Microorganisms Associated with Uranium Bioremediation in a High-Salinity Subsurface Sediment. Appl Environ Microbiol. 69(6): 3672- 3675.

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